**GENOTOXIC STUDIES PERFORMED AFTER RADIOFREQUENCY RADIATION EXPOSURE**

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**Abstract**

**Objective:** With the development of technology human beings are increasingly under the exposure of electromagnetic fields, mainly radio-frequency radiation (RFR) from wireless technologies, mobile phones, base stations etc. There are many genotoxic effects, i.e. DNA and chromosome change studies related RFR exposures. There is still some uncertainty, no definitive conclusions have been reached so far. The aim of present study is to evaluate the genotoxicity studies about RFR exposure reported from 2016 to 1989.

**Material Methods:** The PubMed database from 2016 to 1989 was searched for “radio-frequency radiation” and “genotoxicity tests”. Genotoxicity studies performed under RFR exposure were selected and classified as reported significant effects and reported no significant effect.

**Results:** There were 53 genotoxic effects of RFR studies totally and 19 of them reported genotoxic effects (35,8 %) and 34 of total studies reported no significant effect (64,2 %).

**Coclusion:** It is apparent that there is no consistent pattern that RFR exposure could induce genetic damages. However, one can conclude that under certain conditions of exposure, RFR could be genotoxic. Generally the genotoxic effect of short-term exposure to RFR have been studied up to date. Long-term exposure to RFR is increasing in the environment. So, long-term effect of repeated exposure to RFR should also be studied by taking into consideration the DNA repair processes in order to identify the biological mechanisms involved.

***Keywords:*** *Radio-frequency radiation (RFR), genotoxicity tests, comet assay, chromosome*

 *aberration, sister chromatid exchange, micronuclei*

**Özet**

**Amaç:** Teknolojinin gelişimi ile birlikte, kablosuz teknolojiler, cep telefonları, baz istasyonları gibi kaynaklardan yayılan radyo frekansı radyasyona (RFR) her geçen gün daha çok maruz kalınmaktadır. RFR’ın genotoksik etkileri vardır; DNA ve kromozom değişimleri gibi önemli genotoksik etkilere neden olabilmektedir. Bu çalışmanın amacı, 2016-1989 yılları arasında bildirilen RFR maruziyeti ile ilgili genotoksisite çalışmalarını değerlendirmektir.

**Materyal - Metod:** 2016 - 1989 arasındaki PubMed veritabanı "radyo frekansı radyasyon" ve "genotoksisite testleri" için araştırılmıştır. RFR maruziyeti altında yapılan genotoksisite çalışmaları seçilmiş ve bildirilen önemli etkiler olarak sınıflandırılmıştır.

**Bulgular:** Toplam RFR çalışmalarında 53 genotoksik etki çalışması mevcuttu; bunların 19'unda genotoksik etkiler (% 35,8) bildirmiş ve 34'ünde anlamlı bir etki bildirilmemiştir (% 64,2).

**Sonuç:** RFR'ye maruz kalmanın genetik hasar oluşturabileceğine dair tutarlı bir model bulunmadığı açıktır. Bununla birlikte, belirli maruz kalma koşulları altında RFR'nin genotoksik olabileceği sonucuna varılabilir. Genellikle RFR'ye kısa süreli maruz kalmanın genotoksik etkisi bugüne kadar incelenmiştir. Günümüz insanları her geçen gün daha fazla RFR'ye uzun süreli maruz kalmaktadır. Bu nedenle, tekrarlayan RFR'ye maruz kalmanın uzun vadeli etkisi, ilgili biyolojik mekanizmaları tanımlamak için DNA onarım süreçleri göz önüne alınarak incelenmelidir.

***Anahtarkelimeler:*** *Radyo-frekans radyasyon (RFR), genotoksisite testler, comet assay, kromozom aberasyon, kardeş kromatit değişimi, mikroçekirdek*

**INTRODUCTION**

Radio-frequency radiation (RFR) exposure is available in almost all areas of daily life due to mobile phones, televisions, microwave ovens, base stations, etc. A series of experiments have been performed to investigate possible effects particularly genetic effects of exposure to RFR. There are at least several hundred published papers that report electromagnetic fields (EMF) can affect cellular oxidative processes, can lead to oxidative damage. Increased free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure.

Toxicity to the genome can lead to a change in cellular functions, cancer, and cell death. The ‘comet assay’, also known as the ‘single-cell gel electrophoresis assay’ is the most frequently used technique to study RFR-induced DNA strand breaks. The alkaline comet assay is used to measure single-strand breaks and alkali-labile sites in the DNA molecule. Studies have been carried out to investigate chromosomal conformation and micronucleus formation in cells after exposure to EMF (1). RFR exposures many studies have been conducted regarding alleged health effects but there is still some uncertainty and no definitive conclusions have been reached so far (2). The aim of present study is to evaluate the genotoxic effects of RFR exposure studies reported were from 1989 to 2013.

**MATERIAL METHODS**

PubMed database was searched for the keywords “Radiofrequency radiation” and “genotoxicity” from 2016 to 1989. Genotoxic effects of RFR studies were selected and classified as reported effects and reported no effect.

**RESULTS**

There were 53 genotoxic effects of RFR studies totally and 19 of them reported genotoxic effects (35,8 %) and 34 of total studies reported no significant effect (64,2 %).

**Genotoxic studies that reports effects**

Gandhi et al. (2015) have shown that the study participants comprised 63 persons with residences near a mobile phone tower, and 28 healthy controls matched for gender, age, alcohol drinking and occupational sub-groups. Genetic damage parameters of DNA migration length, damage frequency (DF) and damage index were significantly (p = 0.000) elevated in the sample group compared to respective values in healthy controls. The female residents (n = 25) of the sample group had significantly (p = 0.004) elevated DF than the male residents (n = 38). The linear regression analysis further revealed daily mobile phone usage, location of residence and power density as significant predictors of genetic damage (3). In 2013, Atlı Şekeroğlu et al. investigated the cytogenotoxic effects of 900 MHz RFR and the effect of a recovery period after exposure to RFR on bone marrow cells of immature and mature rats. They exposed 900 MHz RFR to immature and mature rats with electric field levels 28,1 ± 4,8 V/m and 20,0 ± 3,2 V/m. Specific absorption rate (SAR) values were in the range of 0.38-0.78 W/kg, and 0.31-0.52 W/kg 2 h/day for 45 days. Significant differences were observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCE) in all treatment and recovery groups (4). Liu et al. (2013) conducted a 24h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals at SAR of 1 W/kg, 2 W/kg or 4 W/kg. Through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, they determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α-tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. They concluded, findings may imply the novel possibility that RFR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells (5).

Jiang et al.(2012) examined whether such AR could be induced in mice exposed to RFR. Mice were pre-exposed to 900 MHz RFR at 120 µW/cm2 power density for 4 hours/day for 1, 3, 5, 7 and 14 days and then subjected to an acute dose of 3 Gy γ-radiation. The primary DNA damage in the form of alkali labile base damage and single strand breaks in the DNA of peripheral blood leukocytes was determined using the alkaline comet assay. The results indicated that the extent of damage in mice which were pre-exposed to RFR for 1 day and then subjected to γ-radiation was similar and not significantly different from those exposed to γ-radiation alone. However, mice which were pre-exposed to RFR for 3, 5, 7 and 14 days showed progressively decreased damage and was significantly different from those exposed to γ-radiation alone. This data indicated that RFR pre-exposure is capable of inducing AR (6). [Çam](http://www.ncbi.nlm.nih.gov/pubmed?term=%C3%87am%20ST%5BAuthor%5D&cauthor=true&cauthor_uid=22348707) and [Seyhan N](http://www.ncbi.nlm.nih.gov/pubmed?term=Seyhan%20N%5BAuthor%5D&cauthor=true&cauthor_uid=22348707) (2012) collected hair samples from eight healthy human subjects immediately before and after using a 900-MHz GSM mobile phone for 15 and 30 min. Single-strand DNA breaks of hair root cells from the samples were determined using the 'comet assay'. They concluded that a short-term exposure (15 and 30 min) to RFR (900-MHz) from a mobile phone caused a significant increase in DNA single-strand breaks in human hair root cells located around the ear at which is used for the phone communications (7). [Karaca](http://www.ncbi.nlm.nih.gov/pubmed?term=Karaca%20E%5BAuthor%5D&cauthor=true&cauthor_uid=21732071) et al.(2012 ) studies the possible effects of RFR on brain cell cultures of a newborn mouse. Brain cell cultures were exposed to 10.715 GHz with SAR 0.725 W/kg for 6 h in 3 days at 25°C to check for the micronucleus (MNi) assay and the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures that exposed to RFR. They concluded that cell phones which spread RFR may damage DNA and change gene expression in brain cells (8). Sannino et al. (2011) proved the influence of cell cycle on the adaptive response (AR) induced by the exposure of human blood lymphocytes to RFR. Human peripheral blood lymphocytes in G(0)-, G(1)- or S-phase of the cell cycle were exposed for 20 hours to an adaptive dose (AD) of 900 MHz RFR at an average SAR of 1.25 W/kg. Then they were treated with a challenge dose (CD) of 100 ng/ml mitomycin C (MMC). Un-exposed and sham-exposed controls as well as cells treated with MMC alone were included in the study. The incidence of micronuclei (MN) was evaluated to determine the induction of AR. Study confirmed the observations reported in their previous investigation where AR was observed in human blood lymphocytes exposed to AD of RF in S-phase of the cell cycle and further suggested that the timing of AD exposure of RF is important to elicit AR (9). The genotoxic potential of 3 T MRI scans for 22, 45, 67, and 89 min in cultured human lymphocytes was investigated by analyzing chromosome aberrations (CA), micronuclei (MN), and single-cell gel electrophoresis ([Lee JW](http://www.ncbi.nlm.nih.gov/pubmed?term=Lee%20JW%5BAuthor%5D&cauthor=true&cauthor_uid=21412810) et al., 2011). Researchers observed a significant increase in the frequency of single-strand DNA breaks following exposure to a 3 T MRI. In addition, the frequency of both CAs and MN in exposed cells increased in a time-dependent manner. These results suggest that exposure to 3 T MRI induces genotoxic effects in human lymphocytes (10). (Zhijian et al., 2010) A comet assay was used to determine whether 1.8-GHz RFR with SAR of 2W/kg can influence DNA repair in human B-cell lymphoblastoid cells exposed to doxorubicin (DOX). The results demonstrated that RFR could not directly induce DNA damage of human B-cell lymphoblastoid cells; DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect relationship, and there were special repair characteristics of DNA damage induced by DOX; E-E-E type combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX (11). Yao et al. (2008) examined whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz RFR of the Global System for Mobile Communications (GSM). They used a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with SAR of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. After exposure to 1.8 GHz RFR for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RFR at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups (12). Yao K et al. (2008) studied the influence of the 1.8-GHz RFR of the Global System for Mobile Communications on DNA damage, intracellular reactive oxygen species (ROS) formation, cell cycle, and apoptosis in human lens epithelial cells (hLECs). After 24-hour intermittent exposure at SAR of 1 W/kg, 2 W/kg, 3 W/kg, and 4 W/kg, the DNA damage of hLECs was examined by alkaline comet assay. DNA damage examined by alkaline comet assay was significantly increased after 3 W/kg and 4 W/kg radiation (P < 0.05), whereas the double-strand breaks (DSBs) evaluated by gamma H2AX foci were significantly increased only after 4 W/kg radiation (P < 0.05) (13). In 2008 [Schwarz C](http://www.ncbi.nlm.nih.gov/pubmed?term=Schwarz%20C%5BAuthor%5D&cauthor=true&cauthor_uid=18278508) et al. studied human cultured fibroblasts of three different donors; three different short-term human lymphocyte cultures were exposed to 1,950 MHz RFR with SAR of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. [See comment in PubMed Commons below](http://www.ncbi.nlm.nih.gov/pubmed/?term=Int+J+Radiat+Biol.+2011+Sep%3B87(9)%3A993-9#comments)Universal Mobile Telecommunication System (UMTS) exposure increased the comet tail factor (CTF) and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. UMTS exposure may cause genetic alterations in some but not in all human cells in vitro (14). Trosic et al. (2006) exposed rats 2 h/day, 7 days/week to 2450-MHz microwaves at a whole-body SAR of 1.25 +/- 0.36W/kg. Control animals were included in the study. Bone marrow micronucleus frequency was increased on experimental day 15, and polychromatic erythrocytes micronucleus frequency in the peripheral blood was increased on day 8 (15). Banhong et al (2005) was performed to study the synergistic DNA damage effects in human lymphocytes induced by 1.8 GHz RFR, SAR of 3 W/kg with four chemical mutagens, mitomycin C, bleomycin, methyl methanesulfonate, and 4-nitroquinoline-1-oxide. DNA damage of lymphocytes exposed to RFR and/or with chemical mutagens was detected at two incubation time (0 or 21 h) after treatment with comet assay in vitro. The experimental results indicated 1.8 GHz RFR (SAR, 3 W/kg) for 2h did not induce the human lymphocyte DNA damage effects in vitro, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4NQO (16). Busljeta et al. (2004) exposed male rats to 2.45 GHz RFR fields for 2 h/day, 7 days/week, at 5-10 mW/cm2 for up to 30 days. Erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood on irradiation days 8 and 15. Anuclear cells and erythropoietic precursor cells were significantly decreased in the bone marrow on day 15, but micronucleated cells were increased (17).Trosic et al. (2004) investigated increased micronucleus frequency in bone marrow red cells of rats exposed to a 2450-MHz continuous–wave microwaves for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm2 (whole body SAR 1.25 +/- 0.36 (SE) W/kg) (18). Trosic et al. (2002) exposed adult male Wistar for 2 h a day, 7 days a week for up to 30 days to continuous 2450-MHz microwaves at a power density of 5-10mW/cm2. Frequency of micronuclei in polychromatic erythrocytes showed a significant increase in the exposed animals after 2, 8 and 15 days of exposure compared to sham-exposed control (19). Garaj-Vrhovac et al. (1999) examined peripheral blood lymphocytes of 12 subjects occupationally exposed to microwave radiation and showed an increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls (20).

**Genotoxicity tests studies that reported no significant effects**

# [Zhu](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zhu%20K%5BAuthor%5D&cauthor=true&cauthor_uid=27079842) et al.(2016) have shown that extremely low frequency magnetic fields do not induce DNA damage in human lense Epithelial cells in vitro. Human LECs were exposed or sham-exposed to a 50 Hz ELF MF which produced by a well-designed exposure system at the intensity of 0.4 mT. DNA damage in human LECs was examined by the phosphorylated form of histone variant H2AX (γH2AX) foci formation assay and further explored with western blot, flow cytometry, and alkaline comet assay (21). Kumar et al. (2015) reported reported that 900/1800 MHz continuous wave (CW)  radiofrequency radiation (RFR) exposure 2/10 and 2.5/12.4 W/kg specific absorption rate SAR  had no significant effect on the hematopoietic system of rats in the bone marrow (22). Speit et al. (2013) reported no genotoxic effects of RFR in human lymphoblastoid cell line HL-60 cells. Genotoxic effects of RFR were measured by means of the comet assay and the micronucleus test (23). Vijayalaxmi et al. (2013) collected peripheral blood samples from four healthy volunteers and aliquots were exposed in vitro for 2 h to either (i) modulated or unmodulated continuous wave (CW) 2450 MHz RFR with SAR of 10.9 W/kg. Aliquots of the same samples that were exposed in vitro to an acute dose of 1.5 Gy ionizing gamma-radiation (GR) were used as positive controls. The results indicated the following: (i) the incidence of MN was similar in incubator control and those exposed to RFR/sham and Melatonin alone; (ii) there were no significant differences between WCDMA and CW- RFR exposures; (iii) positive control cells exposed to GR alone exhibited significantly increased MN; and (iv) Melatonin treatment had no effect on cells (24). Waldmann et al. (2013) studied the possible genotoxic effect of RFR (GSM, 1,800 MHz) in human lymphocytes, it was investigated by a collaboration of six independent institutes (institutes a, b, c, d, e, h). Peripheral blood of 20 healthy, nonsmoking volunteers of two age groups (10 volunteers 16-20 years old and 10 volunteers 50-65 years old) was taken, stimulated and intermittently exposed to three SARs of RFR (0.2 W/kg, 2 W/kg, 10 W/kg) and sham for 28 h. Four genotoxicity tests with different end points were conducted: chromosome aberration test, micronucleus test, sister chromatid exchange test and the alkaline comet assay. On the basis of these specifications, none of the nine end points tested for SAR trend showed a significant and reproducible exposure effect (25). Jiang et al. (2013) used adult male ICR mice which were pre-exposed to non-ionizing RFR, 900 MHz at 120μW/cm2 power density for 4h/day for 7 days (adaptation dose, AD) and then subjected to an acute whole body dose of 3Gy γ-radiation (challenge dose, CD). The classical micronucleus (MN) assay was used to determine the extent of genotoxicity in immature erythrocytes in peripheral blood and bone marrow.The results indicated that in both tissues, the MN indices were similar in un-exposed controls (26). Ros-Llor et al. (2012) examined effect of mobile phones on micronucleus frequency in human exfoliated oral mucosal cells, they collected two cell samples from each subject, corresponding to the right and left cheek mucosa and found no statistically significant changes in relation to age, gender, body mass index, or smoking status (27). Trosić et al. (2011) evaluated DNA damage in rat's renal, liver and brain cells after in vivo exposure to RFR with a single cell gel electrophoresis/comet assay. Wistar rats were exposed to 915 MHz RFR with power density of 2.4 W/m2, SAR of 0.6 W/kg. The animals were irradiated for one hour/day, 7 days/week during two weeks. The results suggest that, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage (28). Sannino et al. (2009) investigated DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to RFR at 900 MHz. RFR exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). To evaluate DNA damage after RFR exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RFR exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line (29). Zhijian et al. (2009) used in vitro comet assay was used to determine whether 1.8-GHz RFR can influence DNA repair in human leukocytes exposed to X-rays. The SAR of 2 W/kg was applied. The leukocytes from four young healthy donors were intermittently exposed to RFR for 24 h (fields on for 5 min, fields off for 10 min), and then irradiated with X-rays at doses of 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage to human leukocytes was detected using the comet assay at 0, 15, 45, 90, 150 and 240 min after exposure to X-rays. Using the comet assay, the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that the DNA repair speeds of human leukocytes after X-ray exposure exhibited individual differences among the four donors and the intermittent exposures of 1.8-GHz RFR at the SAR of 2 W/kg for 24 h did not directly induce DNA damage or exhibit synergistic effects with X-rays on human leukocytes (30). Luukkonen et al (2009) investigated the effects of 872 MHz RFR on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5 W/kg). The experiments also involved combined exposure to RF radiation and menadione, a chemical inducing intracellular ROS production and DNA damage. No effects of the GSM signal were seen on either ROS production or DNA damage (31). [Kim](http://www.ncbi.nlm.nih.gov/pubmed?term=Kim%20JY%5BAuthor%5D&cauthor=true&cauthor_uid=18214898) et al. (2008) studied in vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) RFR using the alkaline comet assay and chromosomal aberration test. No direct cytogenetic effect of 835-MHz RFR was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RFR alone to damage DNA were nearly negative, although showing a small increase in tail moment (32). Zeni et al. (2008) investigated genotoxic effects in human leukocytes of 1950 MHz with SAR of 2.2 W/kg. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RFR exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays. The results indicate that intermittent exposures of human lymphocytes in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics (33). Juutilainen et al. (2007) examined genotoxicity of long-term exposure to RFR by measuring micronuclei in erythrocytes. In study A, female CBA/S mice were exposed for 78 weeks (1.5 h/d, 5 d/week) to either a continuous 902.5 MHz SAR of 1.5 W/kg. The results did not show any effects of RFR on micronucleus frequency in polychromatic or normochromatic erythrocytes (34). [Chauhan](http://www.ncbi.nlm.nih.gov/pubmed?term=Chauhan%20V%5BAuthor%5D&cauthor=true&cauthor_uid=17214515) et al. (2007) examined non-thermal RFR exposure effects in a series of human-derived cell lines (TK6, HL60 and Mono-Mac-6). Exponentially growing cells were exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RFR for 6 h at mean SAR of 0, 1 and 10 W/kg. The cell culture supernatants were assessed for the presence of a series of human inflammatory cytokines (TNFA, IL1B, IL6, IL8, IL10, IL12) using a cytometric bead array assay. No detectable changes in cell viability, cell cycle kinetics, incidence of apoptosis, or cytokine expression were observed in any of RF-field-exposed groups in any of the cell lines tested, relative to the sham controls (35). Lixia et al. (2006) investigated the DNA damage, expression of heat shock protein 70 (Hsp70) and cell proliferation of human lens epithelial cells (hLEC) after exposure to the 1.8 GHz RFR. An Xc-1800 RFR exposure system was used to employ a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the output power in the SAR of 1, 2 and 3 W/kg. The results indicate that exposure to non-thermal dosages of RFR for wireless communications can induce no or repairable DNA damage and the increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate. The non-thermal stress response of Hsp70 protein increase to RFR exposure might be involved in protecting hLEC from DNA damage and maintaining the cellular capacity for proliferation (36). Scarfi et al [2006] exposed human peripheral blood lymphocytes to 900 MHz GSM signal at SAR of 0, 1, 5 and 10 W/kg peak values. No significant change in micronucleus frequency was observed (37). [Stronati](http://www.ncbi.nlm.nih.gov/pubmed?term=Stronati%20L%5BAuthor%5D&cauthor=true&cauthor_uid=16782651) et al. (2006) examined the possibility of genotoxicity of RFR applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes. Blood specimens from 14 donors were exposed continuously for 24 h to a GSM basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling. By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation (38). Vershaeve et al. (2006) used long-term exposure (2 hrs/day, 5 days/week for 2 years) of rats with 900 MHz GSM signal at 0.3 and 0.9 W/kg and proved that RFR exposure did not significantly affect levels of DNA strand breaks in cells. Maes et al. (2006) published a series of papers on in vitro genotoxic effects of RFR and interaction with chemicals. Their results show no significant effect. Sakuma et al. (2006) exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs to mobile communication radiation for 2 and 24 hrs. No significant change in DNA strand breaks were observed up to 800 mW/kg (39).

[Maes A](http://www.ncbi.nlm.nih.gov/pubmed?term=Maes%20A%5BAuthor%5D&cauthor=true&cauthor_uid=16481348) et al. (2006) investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. The alkaline comet assay, sister chromatid exchange (SCE) and chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test (40). [Sakuma N](http://www.ncbi.nlm.nih.gov/pubmed?term=Sakuma%20N%5BAuthor%5D&cauthor=true&cauthor_uid=16283663) et al. (2006) DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations.A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Their results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg (41).

[Nikolova T](http://www.ncbi.nlm.nih.gov/pubmed?term=Nikolova%20T%5BAuthor%5D&cauthor=true&cauthor_uid=16116041) et al. (2005) used mouse embryonic stem (ES) cells were as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to RFR simulating GSM signals at 1.71 GHz RFR. Short-term RFR exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF-EMF and RFR on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed (42). Diem et al (2005) exposed human fibroblasts and rat granulosa cells to mobile phone signal with frequency of 1800 MHz, SAR 1.2 or 2 W/kg, with different modulations; during 4, 16 and 24 h; intermittent exposures with 5 min on/10min off or continuous. RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the than continuous exposure. They conclude that the induced DNA damage cannot be based on thermal effects (43). Hook et al. (2004) showed that 24-hr exposure of Molt-4 cells to different modulated RFR did not significantly alter the level of DNA damage. Bisht et al. (2002) examined if RFR induces the formation of micronuclei, C3H 10T(1/2) cells were exposed to 835.62 MHz frequency division multiple access (FDMA) or 847.74 MHz code division multiple access (CDMA) modulated RFR. After the exposure to RFR, the micronucleus assay was performed by the cytokinesis block method using cytochalasin B treatment. The results of this study are not consistent with the possibility that these RFR induce micronuclei (44).

[Bisht KS](http://www.ncbi.nlm.nih.gov/pubmed?term=Bisht%20KS%5BAuthor%5D&cauthor=true&cauthor_uid=11966316) et al. (2002) To determine if radiofrequency (RF) radiation induces the formation of micronuclei, C3H 10T(1/2) cells were exposed to 835.62 MHz frequency division multiple access (FDMA) or 847.74 MHz code division multiple access (CDMA) modulated RF radiation. After the exposure to RF radiation, the micronucleus assay was performed by the cytokinesis block method using cytochalasin B treatment. The results of this study are not consistent with the possibility that these RF radiations induce micronuclei (45).

Vijayalaximi et al. (2001) reported that there was no evidence for the induction of micronuclei in peripheral blood and bone marrow cells of rats exposed for 24h to 2450-MHz RFR at a whole body average SAR of 12 W/kg (46). Li et al. (2001) reported no significant change in DNA strand breaks in murine C3H10T(1/2) fibroblasts after 2 hrs of exposure to 847.74 and 835.02 MHz fields at 3-5 W/kg (47). [Vijayalaxmi](http://www.ncbi.nlm.nih.gov/pubmed?term=Vijayalaxmi%5BAuthor%5D&cauthor=true&cauthor_uid=10761010) et al. (2000) collected human peripheral blood samples from three healthy human volunteers and samples were exposed in vitro to pulsed-wave 2450 MHz RFR for 2 h. The lymphocytes were examined to determine the extent of primary DNA damage (single-strand breaks and alkali-labile lesions) using the alkaline comet assay with three different slide-processing schedules. Under the experimental conditions tested, there is no evidence for induction of DNA single-strand breaks and alkali-labile lesions in human blood lymphocytes exposed in vitro to pulsed-wave 2450 MHz RFR, either immediately or at 4 h after exposure (48). Maes et al. (1997) examined the genetic effects of RFR from mobile communication frequencies (935.2 MHz) alone and in combination with a chemical DNA-damaging agent (mitomycin C). Three cytogenetic endpoints were investigated after in vitro exposure of human whole blood cells. These endpoints were the 'classical' chromosome aberration test, the sister chromatid exchange test and the alkaline comet assay. No direct cytogenetic effect was found (49).

Vijayalaximi et al. (1997) exposed C3H/HeJ mice for 20 h/day, 7 days/week, over 18 months to continuous-wave 2450 MHz RFR at a whole-body average SAR rate of 1.0 W/kg. At the end of the 18 months, peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the presence of micronuclei in polychromatic erythrocytes. The results indicate that the incidence of micronuclei/1,000 polychromatic erythrocytes was not significantly different between groups exposed to RFR and sham-exposed groups (50). Meltz et al. (1987) performed the forward mutation assay at the thymidine kinase locus in L5178Y mouse leukemic cells. The power density was 48.8 mW/cm2 and the measured SAR in this system was 30 W/kg. The conclusions from five different experiments, employing three different concentrations of MMC, were that a) RFR exposure alone, at moderate power levels which resulted in a temperature increase in the cell culture medium of less than 3 degrees C, is not mutagenic; and b) when cells are simultaneously treated with MMC and RFR at these same moderate power levels, the RFR does not affect either the inhibition of cell growth or the extent of mutagenesis resulting from the treatment with the chemical MMC alone (51).

We have two reports which show no statistically significant difference between 1800 MHz

and 2100 MHz RF exposed groups with respect to non-exposed groups (Gurbuz et al. 2014;

Gurbuz et al. 2010) (52,53).

**Conclusion**

The epidemiological evidence for a causal association between cancer and RFR exposure is weak and limited. Animal studies have provided no consistent evidence that exposure to RFR at non-thermal intensities causes or promotes cancer. Extensive in vitro studies have found no consistent evidence of genotoxic potential, but in vitro studies assessing the epigenetic potential of RFR exposure are limited. Overall, a weight-of-evidence evaluation shows that the current evidence for a causal association between cancer and exposure to RFR exposure is weak and unconvincing. However, the existing epidemiology is limited and the possibility of epigenetic effects has not been thoroughly evaluated, so that additional research in those areas will be required for a more thorough assessment of the possibility of a causal connection between cancer and the RFR exposure from mobile telecommunications (54). Comet assay was used in most of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. Another concern is that most of the ‘comet assay’ studies were carried out by experimenters who had no prior experience on the assay. Comet assay is that it is a very sensitive assay and requires great care in performing. Thus, different detection sensitivities could result from different experimenters, even following the same procedures. Other endpoint tests were the 'classical' chromosome aberration test, the sister chromatid exchange test (1). Studies have also been carried out to investigate chromosomal conformation and micronucleus formation in cells after exposure to RFR. Attention is also paid to combined exposures of RFR with chemical or physical agents. Again, however, no entirely consistent picture emerges. Many of the positive studies may well be due to thermal exposures, but a few studies suggest that biological effects can be seen at low levels of exposure. Overall, however, the evidence for low-level genotoxic effects is very weak (2). A drawback in the interpretation and understanding of experimental data from bioelectromagnetic research is that there is no general acceptable mechanism on how RFR affects biological systems. The mechanism by which RFR causes genetic effect is unknown. Since the energy level is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced-chemical changes in the cell (1).

Our literature survey, since only 35.8 % of the studies reported effects, it is apparent that there is no consistent pattern that RFR exposure could induce genetic damages/changes in cells and organisms. However, one can conclude that under certain conditions of exposure, RFR is genotoxic.

**Conflict of Interest**

There is no conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence this work (“nothing to declare”).

All authors have materially participated in the research or in the article preparation.

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[See comment in PubMed Commons below](http://www.ncbi.nlm.nih.gov/pubmed/?term=Mutat+Res.+1997+Sep+18%3B393(1-2)%3A151-6.#comments)[Mutat Res.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Mutat+Res.+1997+Sep+18%3B393(1-2)%3A151-6.) 1997; 393(1-2):151-156. **50.** [Vijayalaxmi](https://www.ncbi.nlm.nih.gov/pubmed/?term=Vijayalaxmi%5BAuthor%5D&cauthor=true&cauthor_uid=9092931), [Frei MR](https://www.ncbi.nlm.nih.gov/pubmed/?term=Frei%20MR%5BAuthor%5D&cauthor=true&cauthor_uid=9092931), [Dusch SJ](https://www.ncbi.nlm.nih.gov/pubmed/?term=Dusch%20SJ%5BAuthor%5D&cauthor=true&cauthor_uid=9092931), [Guel V](https://www.ncbi.nlm.nih.gov/pubmed/?term=Guel%20V%5BAuthor%5D&cauthor=true&cauthor_uid=9092931), [Meltz ML](https://www.ncbi.nlm.nih.gov/pubmed/?term=Meltz%20ML%5BAuthor%5D&cauthor=true&cauthor_uid=9092931), [Jauchem JR](https://www.ncbi.nlm.nih.gov/pubmed/?term=Jauchem%20JR%5BAuthor%5D&cauthor=true&cauthor_uid=9092931).Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation. 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**Table 1. Radiofrequency radiation (RFR) exposure and genotoxicity tests studies that reported effects** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Studies Exposure RF- EMF SAR Genotoxicity tests Sample \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Gandhi et al. (2015) (3) 63 persons mobile phone tower DNA migration length

Atlı Şekeroğlu et al. (2013) immature and mature rats 900 MHz (RF-EMF) 0.38-0.78 W/kg, and Chromosome (PCE)

 (4) 0.31-0.52 W/kg 2 h/day aberrations (CA),

 for 45 days Micronucleus (MN)

Liu et al. (2013) (5) mouse spermatocyte-derived 1800 MHz (GSM) signals 1W/kg, 2W/kg or 4W/kg Comet assay GC-2 cell line

 GC-2 cell line 24h intermittent exposure DNA adduct

 (5 min on and 10 min off) DNA strand breaks

[Jiang B](http://www.ncbi.nlm.nih.gov/pubmed?term=Jiang%20B%5BAuthor%5D&cauthor=true&cauthor_uid=22389679) et al. (2012) (6) Mice 900 MHz RF at 120 µW/cm(2) Alkaline comet assay peripheral

 power density for 4 hours/day blood

 for 1, 3, 5, 7 and 14 days and then. leukocytes

 subjected to an acute dose of

 3 Gy γ-radiation

[Çam ST](http://www.ncbi.nlm.nih.gov/pubmed?term=%C3%87am%20ST%5BAuthor%5D&cauthor=true&cauthor_uid=22348707) and Hair samples 900-MHz GSM mobile phone Comet assay human hair

[Seyhan N](http://www.ncbi.nlm.nih.gov/pubmed?term=Seyhan%20N%5BAuthor%5D&cauthor=true&cauthor_uid=22348707) (2012) (7) for 15 and 30 min. DNA single- root cells

 strand break

[Karaca E](http://www.ncbi.nlm.nih.gov/pubmed?term=Karaca%20E%5BAuthor%5D&cauthor=true&cauthor_uid=21732071) et al. (2012) (8) Brain cell cultures of the mice 10.715 GHz 0.725 W/kG signals Micronucleus (MN) Brain cells

 for 6 h in 3 days expression of 11

 proapoptotic

 and antiapoptotic genes

[Sannino A](http://www.ncbi.nlm.nih.gov/pubmed?term=Sannino%20A%5BAuthor%5D&cauthor=true&cauthor_uid=21557704) et al. (2011) (9) Human peripheral 900 MHz RF + 1.25 W/kg Micronuclei (MN) Human

 blood lymphocytes 100 ng/ml 20 hours peripheral

 in G(0)-, G(1)- or mitomycin C blood

 S-phase of the cell cycle lymphocytes

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Studies Exposure RF- EMF SAR Genotoxicity tests Sample \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

[Lee JW](http://www.ncbi.nlm.nih.gov/pubmed?term=Lee%20JW%5BAuthor%5D&cauthor=true&cauthor_uid=21412810) et al. (2011) (10) Cultured human lymphocytes 3 T clinical MRI scans chromosome aberrations (CA), Human

 for 22, 45, 67, and 89 min. micronuclei (MN), lymphocytes

 single-cell gel electrophoresis,

 single-strand DNA breaks

[Zhijian C](http://www.ncbi.nlm.nih.gov/pubmed?term=Zhijian%20C%5BAuthor%5D&cauthor=true&cauthor_uid=19833226) et al. (2010) (11) Human B-cell lymphoblastoid cells 1.8-GHz RFR + 2W/kg comet assay Human B-cell

 Doxorubicin lymphoblastoid

[Yao K](http://www.ncbi.nlm.nih.gov/pubmed?term=Yao%20K%5BAuthor%5D&cauthor=true&cauthor_uid=18509546) et al. (2008) (12) Cultured human lens epithelial cells 1.8 GHz RF GSM 3 W/kg and 4 W/kg alkaline comet assay human lens epithelial

 single strand breaks cells

[Yao K](http://www.ncbi.nlm.nih.gov/pubmed?term=Yao%20K%5BAuthor%5D&cauthor=true&cauthor_uid=18436834) et al. (2008) (13) human lens epithelial cells 1.8 GHz RF GSM 1 W/kg, 2 W/kg, 3 W/kg, alkaline comet assay, human lens epithelial

 and 4 W/kg double-strand breaks cells

 immunofluorescence

 microscope

 apoptosis

[Schwarz C](http://www.ncbi.nlm.nih.gov/pubmed?term=Schwarz%20C%5BAuthor%5D&cauthor=true&cauthor_uid=18278508) et al. (2008) (14) Human cultured fibroblasts 1,950 MHz UMTS 2 W/kg alkaline comet assay, Human fibroblast

 micronucleus

[Trosic I](http://www.ncbi.nlm.nih.gov/pubmed?term=Trosic%20I%5BAuthor%5D&cauthor=true&cauthor_uid=16410191) and [Busljeta I](http://www.ncbi.nlm.nih.gov/pubmed?term=Busljeta%20I%5BAuthor%5D&cauthor=true&cauthor_uid=16410191) (2006) Rats polychromatic erythrocytes (RF/MW) 2.45 GHz 1.25+/-0.36 W/kg micronucleated PCE, BM

(15) (PCEs), bone marrow (BM) 2h/day, 7 days/week

[Baohong W](http://www.ncbi.nlm.nih.gov/pubmed?term=Baohong%20W%5BAuthor%5D&cauthor=true&cauthor_uid=15935405) et al. (2005) (16) human lymphocytes 1.8 GHz RFR + 3 W/kg comet assay human lymphocytes

 mitomycin C,

 bleomycin,

 MMS,

 4NQO

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Studies Exposure RF- EMF SAR Genotoxicity tests Sample \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

[Busljeta I](http://www.ncbi.nlm.nih.gov/pubmed?term=Busljeta%20I%5BAuthor%5D&cauthor=true&cauthor_uid=15729835) et al. (2004) (17) erythropoietic changes in rats (RF/MW) irradiation 5-10 mW/cm2 micronucleus (MN) peripheral blood

 at nonthermal level bone marrow

 2.45 GHz continuous

 RF/MW fields for

 2 hours daily,7 days a week,

[Trosic I](http://www.ncbi.nlm.nih.gov/pubmed?term=Trosic%20I%5BAuthor%5D&cauthor=true&cauthor_uid=15388808) et al. (2004) (18) bone marrow red cells of rats 2.45 GHz continuous RF/MW 1.25 +/- 0.36 (SE)W/kg micronuclei PCEs bone marrow

 field for 2 h daily, 7 days a week

[Trosic I](http://www.ncbi.nlm.nih.gov/pubmed?term=Trosic%20I%5BAuthor%5D&cauthor=true&cauthor_uid=12438005)et al. (2002) (19) Wistar rats (PCEs) 2,450 MHz (rf/MW) 5-10 mW/cm(2) micronuclei PCEs Peripheral blood

 2 h a day, 7 days a week

 for up to 30 days

[Garaj-Vrhovac V](http://www.ncbi.nlm.nih.gov/pubmed?term=Garaj-Vrhovac%20V%5BAuthor%5D&cauthor=true&cauthor_uid=10576101) (1999) (20) peripheral blood lymphocytes (RFR) (MW) micronuclei (MN) lymphocytes